6 DEDICATED PCR FACILITIES

To minimize the potential for carry-over contamination, the laboratory must be organized so that the area in which amplified DNA is handled is physically isolated in a separate room from the work areas for DNA extraction and PCR setup. The laboratory will have designated work areas for evidence handling, DNA extraction, and PCR setup. (These may be in the same room, but should be clearly delineated). Another area is reserved strictly for PCR amplification and typing, and other activities that require handling of amplified DNA.

NOTE: In order to trace the order in which the samples were processed/handle the sample will be listed on the worksheet in the order in which they were processed/handled.

If contamination is identified which is not isolated to a single case and could adversely affect all of the DNA analyses conducted in the section, the supervisor will be notified immediately. Subsequently, the supervisor will notify the Forensic Biology Section Chief of the contamination so that assistance can be provided to correct the problem and to ensure that the incident is properly documented. Depending on the level of contamination and severity, if necessary the Forensic Biology Section in the affected laboratory will be shut down until the problem can be corrected. In order to prevent compromising evidence samples, if the Forensic Biology Section Chief is not available and therefore the problem cannot be discussed, if the level of contamination is wide spread, the supervisor has the discretion to shut down the section to correct the problem once he/she has notified the laboratory director and the Forensic Biology Section Program Manager.

6.1 DNA Extraction Work Area

This work area is used only for evidence handling and for extraction and isolation of DNA. Microscopy, photography, and any other activities that involve the handling of evidence samples before amplification may also be performed in this work area.

6.1.1 Equipment and supplies used in the DNA extraction work area are exposed to relatively high concentrations of human genomic DNA, and therefore the following dedicated equipment and supplies should not be used in any other area except the DNA extraction work area.

- Pipettes: adjustable 2.0 µL, 10 µL, 20 µL, 100 µL, 200 µL and 1000 µL
- Repeater pipette
- Microcentrifuge tube racks
- Microcentrifuge tubes (1.5 mL)
- Microcentrifuge
- Scissors
- Scalpel with disposable blades
- Sterile plugged pipette tips
- Slot-blot apparatus
- Horizontal gel electrophoresis apparatus
- Power supply
- Refrigerator/Freezer
- Sterile laboratory glassware
- Disposable gloves
- Lab coats
- Forceps
- Kimwipes and Kaydrys

- Vortex
- Heat Block

6.1.2 Special Precautions

6.1.2.1 Perform the DNA extraction and PCR setup of evidence samples at a separate time or space from the DNA extraction and PCR setup of reference samples. This helps to prevent potential cross-contamination between evidence samples and reference samples.

6.1.2.2 Perform DNA extractions of samples containing high levels of DNA (for example, bloodstains) separately from samples containing a low level of DNA (single hairs, small bloodstains, etc.) to minimize the potential for sample-to-sample contamination.

6.1.2.3 Use disposable gloves at all times. Change gloves frequently to avoid sample-to-sample contamination with DNA and whenever exiting the work area. **DO NOT TOUCH** any surface which may contain a contaminant, such as the surface of the skin, eyes, safety glasses, clothing, or a non-cleaned bench-top, while wearing disposable gloves or working with evidence.

6.1.2.4 After cutting each sample thoroughly clean scissors and other implements with 10% bleach (or a solution that will degrade DNA) followed by cleaning with isopropyl alcohol and/or ethanol or use a fresh scalpel blade to cut each item.

6.1.2.5 Use a clean cutting surface for each piece of evidence.

6.1.2.6 Use sterile disposable plugged pipette tips and microcentrifuge tubes.

6.1.2.7 Change pipette tips between handling each sample.

6.1.2.8 Store reagents in small quantities to minimize the number of times a given tube of reagent is opened.

6.1.2.9 To avoid splashing centrifuge all liquid to the bottom of the closed tube before the tube is opened.

6.1.2.10 Include reagent blank controls with each set of DNA extractions to check for the presence of contaminating DNA in the reagents.

6.1.2.11 Thoroughly clean all work surfaces with 10% bleach (or a solution that will degrade DNA) followed by cleaning with isopropyl alcohol and/or ethanol before setting up the DNA Extraction Work Area. Use disposable bench paper or a Kaydry to prevent the accumulation of human DNA on permanent work surfaces. After each set of samples discard the bench paper or Kaydry.

6.1.2.12 Limit the quantity of samples handled during a single analysis to a manageable number. This precaution reduces the risk of sample mix-up and the potential for sample-to-sample contamination.

6.1.2.13 Wear a dedicated lab coat for pre-amplification sample handling when working in the DNA Extraction Work Area.

6.2 PCR Setup Work Area

This work area is used only for adding sample DNA to the PCR Reaction Mix.

6.2.1 To prevent transfer of exogenous human DNA into the PCR setup area, the following dedicated equipment and supplies used for PCR setup should not be taken out of the PCR setup work area. A wipe test of this area is conducted on a monthly basis. (Refer to Appendix H for the procedure.)

- Pipettes: adjustable 10 µL, 20 µL, 200 µL for adding DNA samples to the PCR Reaction Mix
- Repeater pipette
- Sterile plugged pipette tips
- Microcentrifuge tubes and caps
- Microcentrifuge tube rack
- Disposable gloves
- GeneAmp or MicroAmp tubes
- Kimwipes

6.2.2 Special Precautions

6.2.2.1 Use a dedicated 10 µL or 20 µL pipette for adding sample DNA to the PCR Reaction Mix.

6.2.2.2 DNA is always added to the PCR Reaction Mix (i.e., primers, 10X buffer, type 1 water, and DNA polymerase) last. All tubes are capped once the PCR Reaction Mix has been added to the tubes prior to the addition of DNA.

6.2.2.3 After the addition of each DNA sample, re-cap the tube before proceeding to the next sample.

6.2.2.4 Cap the negative amplification control tube last, after all DNA samples have been added to the other tubes. This control provides a check for contamination occurring during PCR setup.

6.2.2.5 Change gloves frequently, whenever they may have been contaminated with DNA.

6.2.2.6 Avoid touching the inside surface of the tube caps.

6.2.2.7 Change pipette tips after addition of each sample DNA to a PCR Reaction Mix.

6.2.2.8 Store the DNA amplification reagents away from amplified product, which serves as a barrier to possible contamination by exogenous DNA.

6.3 Amplified DNA Work Area

This work area is a physically separate area used only for those activities that involve the handling of amplified DNA. This includes DNA typing, gel electrophoresis of amplified DNA, waste disposal of amplified DNA solutions, and storage of amplified DNA. Conduct a wipe test of this area on a monthly basis. (Refer to Appendix H for the procedure.)

6.3.1 Do not remove amplified DNA or the following dedicated equipment and supplies used to handle amplified DNA from the amplified DNA work area.

NOTE: Samples that have not yet been amplified should never come in contact with this equipment.

- Thermal Cycler
- Pipette: adjustable 2 µL, 10 µL, 20 µL, 100 µL and 200 µL
- Disposable gloves
- Kimwipes
- Microcentrifuge tube racks
- Sterile plugged pipette tips
- Gel electrophoresis apparatus
- Power supply
- Ice bucket and ice or freezer pack
- Microcentrifuge tubes and/or microtubes with caps
- Microcentrifuge
- Freezer
- Dedicated glassware and plastic-ware
- Disposable laboratory coats

6.3.2 Special Precautions

Carefully handle amplified DNA to avoid dispersing it around the room. Reducing the dispersal of amplified DNA within this work area reduces the potential for transfer of amplified DNA to other work areas.

6.3.2.1 Always remove gloves prior to leaving the Amplified DNA Work Area to avoid the transfer of amplified DNA into other work areas.

6.3.2.2 Change gloves whenever they become contaminated with amplified DNA to reduce unnecessary dispersal of DNA around the work area.

6.3.2.3 To avoid splashing carefully open tubes containing amplified DNA.

6.3.2.4 Store tubes of amplified DNA in a designated refrigerator or freezer in a separate box from the reagents used during the typing step. Store tubes of amplified DNA in a separate freezer from the amplification reagents.

♦**END**